

AMENDMENTS TO THE CLAIMS

Claims:

1. (Currently Amended) A recombinant HuEPO-L-vFc fusion protein consisting of comprising HuEPO, a peptide linker, and a human IgG Fc variant, wherein the recombinant HuEPO-L-vFc fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.
2. (Currently Amended) The recombinant HuEPO-L-vFc fusion protein of Claim 1, wherein the peptide linker ~~in claim 1~~ containing ~~about~~ 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
3. (Currently Amended) The recombinant HuEPO-L-vFc fusion protein of Claim 1 or Claim 2, wherein the human IgG Fc variant ~~in claim 1 or claim 2 comprising~~ comprises a hinge, CH2, and CH3 domains of human IgG2 with Pro331Ser mutation as of SEQ ID NO: 18.
4. (Withdrawn) ~~The human IgG Fc variant in claim 1 or claim 2 comprising a hinge, CH2, and CH3 domains of human IgG4 with Ser228Pro and Leu235Ala mutations as SEQ ID NO: 20.~~
5. (Withdrawn) ~~The human IgG Fc variant in claim 1 or claim 2 comprising a hinge, CH2, and CH3 domains of human IgG1 with Leu234Val, Leu235Ala, and Pro331Ser mutations as SEQ ID NO: 22.~~

6. (Withdrawn) ~~The HuEPO-L-vFc fusion protein of any of the preceding claims exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.~~
7. (Currently Amended) A CHO-derived cell line transfected with DNA encoding the recombinant ~~producing the~~ HuEPO-L-vFc fusion protein of any of the preceding claims produces in its growth medium in excess of 10 µg per million cells in a 24 hour period.
8. (Original) The CHO-derived cell line producing the HuEPO-L-vFc fusion protein of claim 7 in its growth medium in excess of 30 µg per million cells in a 24 hour period.
9. (Currently Amended) The CHO-derived cell line producing the HuEPO-L-vFc fusion protein of claim 1, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG ~~selected from the group consisting of IgG1 as SEQ ID NO: 22, IgG2 as of SEQ ID NO: 18, and IgG4 as SEQ ID NO: 20, and~~ the IgG Fc contains amino acid mutations to attenuate effector functions, a flexible peptide linker containing about 20 or fewer amino acids is present between HuEPO and human IgG Fc variant, ~~and the HuEPO-L-vFc fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.~~
10. (Currently Amended) A method for making a recombinant fusion protein comprising HuEPO, a flexible peptide linker, and a human IgG Fc variant, ~~which~~ wherein said method comprises: (a) generating a CHO-derived cell line; (b) growing the cell line under conditions wherein the recombinant fusion protein is expressed in its growth medium in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed recombinant fusion protein from step (b), wherein the recombinant fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.

11. (Currently Amended) The method of claim 10, wherein the flexible peptide linker containing about 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
12. (Currently Amended) The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG2 of SEQ ID NO: 18 with Pro331Ser mutation.
13. (Withdrawn) ~~The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG4 with Ser228Pro and Leu235Ala mutations.~~
14. (Withdrawn) ~~The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG1 with Leu234Val, Leu235Ala, and Pro331Ser mutations as SEQ ID NO: 18.~~
15. (Currently Amended) The method of any claim of claims 10, 11, and 12, ~~and 13, and 14~~, wherein step (b) is in excess of 30 µg per million cells in a 24 hour period.
16. (Withdrawn) ~~The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG4 with Ser228Pro and Leu235Ala mutations as SEQ ID NO: 20.~~
17. (Withdrawn) ~~The method of claim 16, wherein step (b) is in excess of 30 µg per million cells in a 24 hour period.~~

18. (Withdrawn) ~~The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG1 with Leu234Val, Leu235Ala, and Pro331Ser mutations as SEQ ID NO: 22.~~
19. (Withdrawn) ~~The method of claim 18, wherein step (b) is in excess of 30 µg per million cells in a 24 hour period.~~
20. (Currently Amended) A method for making a recombinant fusion protein comprising HuEPO, a flexible peptide linker, and a human IgG Fc variant, which method comprises: (a) generating a CHO-derived cell line transfected with DNA encoding the recombinant HuEPO-L-vFC fusion protein; (b) growing the CHO cell line under conditions the recombinant protein is expressed in its growth medium in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed protein from step (b), wherein the recombinant fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis; wherein the flexible peptide linker containing about 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine; wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains selected from the group consisting of human IgG2 with Pro331Ser mutation as of SEQ ID NO: 18, ~~human IgG4 with Ser228Pro and Leu235Ala mutations as SEQ ID NO: 20, and human IgG1 with Leu234Val, Leu235Ala, and Pro331Ser mutations as SEQ ID NO: 22.~~

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Claims:

1. A recombinant HuEPO-L-vFc fusion protein consisting of HuEPO, a peptide linker, and a human IgG Fc variant, wherein the recombinant HuEPO-L-vFc fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.
2. The recombinant HuEPO-L-vFc fusion protein of Claim 1, wherein the peptide linker containing 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
3. The recombinant HuEPO-L-vFc fusion protein of Claim 1 or Claim 2, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG2 with Pro331Ser mutation of SEQ ID NO: 18.
4. (Withdrawn)
5. (Withdrawn)
6. (Withdrawn)
7. A CHO cell line transfected with DNA encoding the recombinant HuEPO-L-vFc fusion protein of any of the preceding claims produces in its growth medium in excess of 10 µg per million cells in a 24 hour period.

8. The CHO-derived cell line producing the HuEPO-L-vFc fusion protein of claim 7 in its growth medium in excess of 30 µg per million cells in a 24 hour period.
9. The CHO-derived cell line producing the HuEPO-L-vFc fusion protein of claim 1, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG2 of SEQ ID NO: 18, the IgG Fc contains amino acid mutations to attenuate effector functions, and a flexible peptide linker containing 20 or fewer amino acids is present between HuEPO and human IgG Fc variant.
10. A method for making a recombinant fusion protein comprising HuEPO, a flexible peptide linker, and a human IgG Fc variant, wherein said method comprises:
(a) generating a CHO-derived cell line; (b) growing the cell line under conditions wherein the recombinant fusion protein is expressed in its growth medium in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed recombinant fusion protein from step (b), wherein the recombinant fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis.
11. The method of claim 10, wherein the flexible peptide linker containing 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine.
12. The method of claim 10, wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains of human IgG2 of SEQ ID NO: 18 with Pro331Ser mutation.
13. (Withdrawn)

14. (Withdrawn)

15. The method of any claim of claims 10, 11, and 12 wherein step (b) is in excess of 30 µg per million cells in a 24 hour period.

16. (Withdrawn)

17. (Withdrawn)

18. (Withdrawn)

19. (Withdrawn)

20. A method for making a recombinant fusion protein comprising HuEPO, a flexible peptide linker, and a human IgG Fc variant, which method comprises: (a) generating a CHO cell line transfected with DNA encoding the recombinant HuEPO-L-vFC fusion protein; (b) growing the CHO cell line under conditions the recombinant protein is expressed in its growth medium in excess of 10 µg per million cells in a 24 hour period; and (c) purifying the expressed protein from step (b), wherein the recombinant fusion protein exhibits *in vitro* biological activity similar to or higher than that of rHuEPO on a molar basis; wherein the flexible peptide linker containing 20 or fewer amino acids is present between HuEPO and the human IgG Fc variant; and the peptide linker comprises two or more amino acids selected from the group consisting of glycine, serine, alanine, and threonine; wherein the human IgG Fc variant comprises a hinge, CH2, and CH3 domains selected from the group consisting of human IgG2 with Pro331Ser mutation of SEQ ID NO: 18.